

ON HUMAN AMYLASE
ISOENZYMES

by
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INTRODUCTION

α -Amylase (E.C. 3.2.1.1, α -1,4-glucan-4-glucanohydrolase) is a Ca^{++} -requiring enzyme, which catalyses the hydrolysis of starch, glycogen, and related poly- and oligosaccharides by a random cleavage of α -D-(1 $\rightarrow$ 4) glucan linkages, converting the substrate to reducing carbohydrate fragments (44). The enzyme does not attack the 1 $\rightarrow$ 6 linkages of amylopectin. The end products formed from amylose are mainly maltose and a minor amount of glucose. The end products formed from amylopectin are maltose, small chain dextrins (limit dextrins), and some glucose (42). In man large amounts of amylase are secreted into the digestive tract by the salivary and exocrine pancreatic glands, minimal amounts being produced also in other tissues (see 36 and below).

The starch degrading activity of serum was discovered as early as 1846 (31) and that of urine in 1863 (12). It was, however, not until the first decade of this century, when Wohlgemuth had introduced his semiquantitative method for determination of "diastase" in urine as a test of pancreatic disease (71), that determination of amylase became a tool in practical medicine. The method of Wohlgemuth was most widely used for determining amylase during the following half century, after which it was replaced by more sophisticated techniques. These methods were either amyloclastic, in which the colour reaction between starch and iodine was taken as an index of starch digestion, or saccharogenic. i.e. the amount of liberated glucose, maltose, or isomaltose was determined. In the last decade new principles, such as the use of chromogenic substrates for the detection of amylase activity, have been developed and largely replaced earlier methods (7,11,25,44,47). These methods are based on the principle that owing to the amylolytic activity small soluble fragments are liberated from an insoluble dye-starch polymer into the reaction solution; the staining intensity being proportional to the amylase activity. The total amylase activity of a serum or urine sample is useful in the diagnosis of a lesion of an organ

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producing amylase. If, however, more than one organ produces amylase, and some of it leaks into the blood stream, the abnormal amylase secretion from one organ may be asked by that of another.

Since the late fifties it has been known that an enzyme often exists in multiple molecular forms (33), which can be separated from each other because of differences in their physico-chemical properties. Many of these isoenzymes are more or less tissue specific. Hence, changes of the normal serum isoenzyme pattern may be diagnostically useful even when the total serum enzyme activity is non-informative. This explains the wide clinical use of isoenzyme separation in serum for lactate dehydrogenase and alkaline phosphatase. So far isoamylase separation is not widely used although it has long been known that it is produced by the exocrine pancreas as well as by the salivary glands.

The diagnosis of pancreatic disease is often difficult in cases of chronic pancreatic disease and carcinoma. The diagnostic methods used include pancreatic scanning, ultrasound investigation, angiography, endoscopic retrograde cholangio-pancreatography, and tests of the exocrine pancreatic secretory ability, which require intestinal intubation. As the latter group of tests, however, often are laborious and inconvenient for the patients, it would be an advantage if the functional state of the pancreas could be assessed from a simple blood sample. Such assessment would require a technique allowing specific detection and estimation of the serum isoamylases from various sources.

HISTORICAL

During the last two decades many papers on the electrophoretic mobility of human amylase have been published (for review see 36). But owing to the wide variety of methods used many of the results are incomparable or conflicting. Electrophoresis has been performed as free electrophoresis (37) or as electrophoresis in various stabilizing media, such as paper (8,14,34,68,69), cellulose acetate (4,13,27,41,46,48,63), agar and agarose (1,5,6,18,20,21,39), or polyacrylamide (16,38,57,64,69,72,73). After separation the amylase activity has been measured in eluates of strips cut out of the electropherograms, of with the aid of zymogram techniques using either the electropherogram itself or a replica transfer of it. The methods for detecting amylase have been either amyloclastic or saccharogenic. These methods are apt to give rise to false positive reactions, proteins, lipoids and unsaturated substances, as well as pH variations being known to interfere with the starch-iodine colour reaction used in the amyloclastic methods (43,56,68) or with the glucose in the saccharogenic methods (19). For instance normal serum amylase has been described as migrating at the same rate as albumin and when using a saccharogenic method for amylase detection it was localized to the place where the glucose of the original blood samples was situated after the electrophoretic separation.

Nørby (39) first convincingly showed that the human salivary and pancreatic isoamylases differed from each other in electrophoretic migration; the salivary amylase migrated faster towards the anode than did the pancreatic amylase. Using a method that normally did not identify more than one salivary and one pancreatic amylase fraction in serum, Kamarýt and Laxová (20,21) were the first to detect an inherited duplication of one or both of these fractions. Abnormal serum isoamylase patterns were also noticed in some diseases: the salivary

amylase fraction in serum was increased in mumps (20,40) while the pancreatic fraction in serum was increased in acute pancreatitis (1,40). Aw and co-workers (4,5,18) separated the isoamylases of concentrated urine and found an increase of the salivary isoamylases in mumps and an increase of the pancreatic isoamylases in acute pancreatitis. They also reported a decreased activity of the pancreatic isoamylase in the urine in chronic pancreatitis (6,18). Using starch-iodine staining for detecting amylase after electrophoresis Taussig et al. (64) found no pancreatic serum isoamylase in two thirds of patients with cystic fibrosis of the pancreas. In Sjögren's syndrome the salivary serum isoamylases are sometimes increased, sometimes normal, and sometimes decreased (72).

In recent years a number of reports have appeared on the variation of human serum isoamylases as detected with insoluble dye-starch polymers i.e. chromogenic substrates after separation of the iso-enzymes (9,13,27,41,46,48,57,63). The use of such substrates has revealed high pancreatic serum isoamylase activity in acute pancreatitis or relapse of chronic pancreatitis and high salivary serum isoamylase activity in disorders of the parotid glands.

The salivary serum isoamylases is occasionally reported increased also in acute pancreatitis (41). Such an increase has also been reported by another group (15), who used a chromatographic method for the separation of salivary and pancreatic serum isoamylases. A specific serum isoamylase pattern in macroamylasemia (70, for review see 36 and 58) was claimed by one group (41), while another group (27) found a normal isoenzyme distribution in serum of a patient with "clinically" diagnosed macroamylasemia.

Using isoelectric focusing on columns to study the serum isoamylases Lewitt et al. (28) reported distinct patterns in affections of the salivary glands and in acute pancreatitis. In patients with

pancreatic exocrine insufficiency they found the activity of the pancreatic serum amylase to be substantially reduced.

The occurrence of multiple forms of salivary amylase was first demonstrated in the early sixties. Generally one main fraction as well as a varying number of additional weak zones of amylase have been reported; the total number of salivary isoamylases being from 1 to 5, or even 8 depending on the techniques used for the separation and detection of isoamylases (for review see 36).

Aw and co-workers (5,6,18) were the first to demonstrate multiple pancreatic amylase fractions, and up to 5 amylolytic zones have been detected in pancreatic juice after electrophoresis (see 36). A genetically determined variation of the isoenzyme pattern of the pancreatic as well as of the salivary isoamylases was first described by Kamarýt & Laxová; the variation was also demonstrable in the serum (20,21 and see 36). It has since been shown that the alleles determining the pancreatic and salivary isoamylase patterns, respectively, are situated in chromosome no 1 and closely linked (for review see 17).

Amylase activity as well as the prevalence of individual isoamylases has also been demonstrated in human milk and in the contents of ovarian cysts (36,65); the isoamylases of ovarian cysts have a high electrophoretic migration rate towards the anode. As for the existence of a human liver amylase contradicting reports have been published (see 36).

Physico-chemical studies of human isoamylases (for review of early reports, see 36) have been performed mostly on salivary isoamylases (22,23) and only occasionally on individually isolated salivary isoamylase fractions (10,34). In brief, on gel filtration the 5 principal salivary isoamylases can be separated into two families, one containing about 5 per cent carbohydrate and the other, none (22,23,34); as for

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the presence of sialic acid opinions differ (10,22,23,34); the molecular weights have been given as 55,000 to 60,000 daltons (23,34); peptide mapping after pepsin digestion has disclosed almost identity for the two families (22), and no significant differences in amino acid composition have been reported (22,34). The pancreatic amylase has been described as possessing no carbohydrate; a molecular weight of about 54,000 daltons; and striking similarities to the salivary isoamylases in peptide pattern and amino acid composition (59). There is a close antigenic relationship between the 2 families of salivary isoamylase and the pancreatic amylase (10,62, and see 36). Various human isoamylases may differ from one another in their capacity to hydrolyse starches from different sources (60, and see 36).

PRESENT INVESTIGATION

Purpose and design

The purpose of the investigation was to elucidate the variation of the isoenzymes of human amylase in health and disease as well as some physico-chemical differences between individual isoamylases.

A routine method for determining serum isoamylase should preferably be simple and cheap. It should also give sufficient qualitative separation as well as reproducible quantitative determination of relevant isoenzyme fractions. As agarose is the most widely used supporting medium for high resolving gel electrophoresis of plasma proteins, lipoproteins, lactate dehydrogenase and alkaline phosphatase isoenzymes, it was decided to use it also for separation of isoamylase.

A semiquantitative immunological method was devised for detecting genetic variants of the isoamylase pattern in saliva (I). For clinical use, however, quantitative determinations of the individual serum isoamylases must be reproducible. As individual isoenzymes may have distinct starch degrading activities on different substrates, the same substrate should be used for the determination of the total activity of the sample as for the specific determination of the individual isoamylases. As the Phadebas[®] Amylase test (Pharmacia, Uppsala, Sweden) is now used routinely for determining serum isoamylase a zymogram technique based on this substrate was worked out (II,III).

The sources of the various detectable serum isoamylases had to be considered and for this purpose various body fluids and tissue homogenates were studied (IV). As there are three different groups of isoamylase, the salivary, the pancreatic, and the genital groups and as all of them contain a number of specific isoenzymes, it was thought worth while to study their physico-chemical relationship (V).

A requisite for valid conclusions from the results obtained in patients required knowledge of the normal quantitative as well as qualitative variation of the isoenzymes studied (III,IV,VII).

The clinical material used in the investigation consisted of patients with affections of the pancreatic and the salivary glands (VI,VII,VIII,IX).

Basic studies

Techniques (I,II,III)

Rabbit anti-human parotid amylase was obtained after immunization of the animals with amylase prepared by gel filtration of parotid saliva. The antiserum also precipitated human pancreatic amylase. With crossed immunoelectrophoresis (26) and immunofixation electrophoresis (2) the amylase of saliva separated into 5 isoenzymes after prolonged electrophoresis in agarose gel using a barbital buffer pH 8.6. Three amylase fractions were identified in pancreatic juice. In concentrated urine, amylase from both sources could be recognized. The immunologic identification of amylase gave the same results as an enzymatic staining procedure using the starch-iodine reaction.

Owing to the small differences in migration rate between the individual isoenzymes the separation was not distinct unless the electrophoresis time was prolonged. Protein staining for the study of salivary isoamylase polymorphism was not satisfactory because of the faint staining of some amylase fractions. In addition, some salivas contained an extra protein fraction in the amylase region. The starch-iodine staining method for detection of amylase activity was found to be too sensitive to variation in enzyme activity of the various isoamylases to be acceptable for routine use. Moreover, a mutation with destruction of the enzymatic properties but not of all the immunologic properties of the amylase molecule could not be excluded.

In the event of such a mutation it would be possible to detect the enzymatically defective protein with an immunologic method. Immuno-fixation electrophoresis was recommended as the method of choice for the routine study of amylase in saliva with supplementary crossed immunoelectrophoresis in selected cases. A drawback of the immunologic identification method was that it could not be used for the study of serum amylase owing to the low enzyme concentrations in the blood (I).

A method based on a commercially available coloured insoluble dye-starch polymer (Phadebas^R Amylase Test) was developed for the detection of the serum isoamylase distribution after prolonged agarose gel electrophoresis. The method excluded the risk of artifacts due to the interaction of proteins in the starch-iodine staining reaction. Agarose was selected as separation medium, and a conventional barbital buffer pH 8.6, identical with the one used for routine separation of plasma proteins was used. The method proved useful in clinical practice. A disadvantage was that the pancreatic isoamylases from persons with genetically determined slow-migrating pancreatic isoamylase fractions remained at the application slot with the result that their activity could not be properly estimated (II).

To solve this problem the technique was modified. The separation was performed at a slightly higher pH, and a linear polyacrylamide polymer was included in the agarose gel to reduce the endosmotic flow. In this system the slowly migrating pancreatic amylase fractions migrated towards the anode. The technique described detected isoamylase fractions of low activity and could easily be modified to disclose individual amylase zones with an activity down to about 1 per cent of normal serum activity (III).

Sources of human serum amylase (IV)

The isoamylases in various tissue homogenates and body fluids were

studied with the new technique. Nothing suggested any significant production of amylase in the liver, but minute amounts of amylase belonging to the pancreatic group of isoamylases might be produced by the glands of the proximal duodenum. The specific group of isoamylases produced in the female genital tract could not be demonstrated in serum and urine, although it has been found in fluid from the cul-de-sac (53). It was concluded that the activity of amylase in serum and urine was derived from two groups of isoenzymes, one group originating from the salivary glands, the other from the pancreas.

Physico-chemical properties of individual isoamylases (V)

Individual isoamylases from parotid saliva and pancreatic juice were isolated by gel filtration and isoelectric focusing. On gel filtration the parotid isoamylases separated into three families of isoenzymes. The individual isoamylases of two of the families could be isolated by isoelectric focusing. The peptide pattern after cyanogen bromide cleavage as well as after digestion by thermolysin showed great similarities between the individual isoamylases. The polypeptide parts of the 5 isolated salivary isoamylases thus seemed to be almost identical. They all also had common antigenic determinants, though some determinants confined to individual isoamylases were also found. Differences in carbohydrate content i.e. the number of neutral hexoses and the number of sialic acid residues, caused some variation of the molecular weights and the isoionic points. The sialic acid-containing side chains were located in one or the other or both of 2 different cyanogen bromide fragments. Neutral hexoses constituted 3 to 6 per cent in the B-isoamylase family, but none were demonstrable in the isolated isoamylases of the C-family. The specific characteristics of the salivary A-isoamylase family, having a varying number of sialic acid residues, could not be disclosed owing to the lowness of its

concentration. The differences between the individual salivary isoamylases may thus be due to differences in the amount of carbohydrate and sialic acid.

The pancreatic group of isoamylases had no carbohydrate and a lower molecular weight but possessed great similarities with the salivary isoamylases in amino acid composition and amino acid sequence, as judged from the polypeptide pattern. Their optimal enzymatic activity was at a slightly higher pH than that of the salivary isoamylases. The individual pancreatic isoenzymes did not differ in sialic acid content and they did not separate on gel filtration.

The isoamylases produced by the endometrium of the isthmus seemed to be closely related to the salivary isoamylases. The fallopian tubes produced these amylases as well as a specific group of tubal isoamylases with lower isoionic points than any of the other groups of isoamylases. The tubal specific isoamylases also varied in their content of sialic acid.

Reference values (IV,VII)

The contribution of the isoamylases from the salivary glands and the pancreatic gland to the total serum amylase activity was determined from early foetal life to adult age. In serum from 14-week old foetuses a very low activity of the salivary isoamylase was regularly found. The activity increased steadily with age and reached the normal adult level, about 80 U/l ($1.5 \mu\text{kat/l}$), at the age of 5 years (Fig. 1). The pancreatic group of isoenzymes in serum developed later; the majority of children below 3 months had no demonstrable pancreatic serum isoamylase activity. The activity rose slowly to reach adult level, about 85 U/l ($1.6 \mu\text{kat/l}$) at the age of 10 to 15 years (Fig. 1).

The salivary serum isoamylase activity tended to be skewly distributed towards low levels (Fig. 2). In contrast, the distribution of the pancreatic serum isoamylase showed a positive skew, but became normally distributed after logarithmic transformation (Fig. 2). The

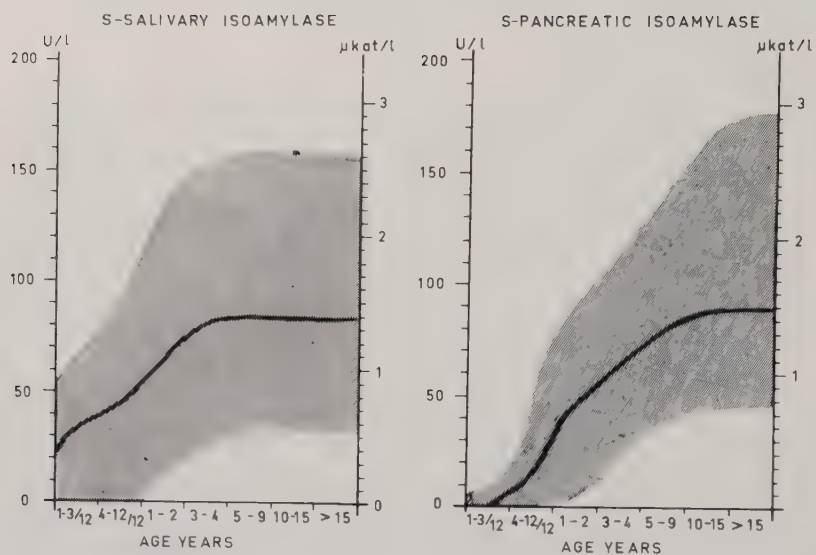


Fig. 1

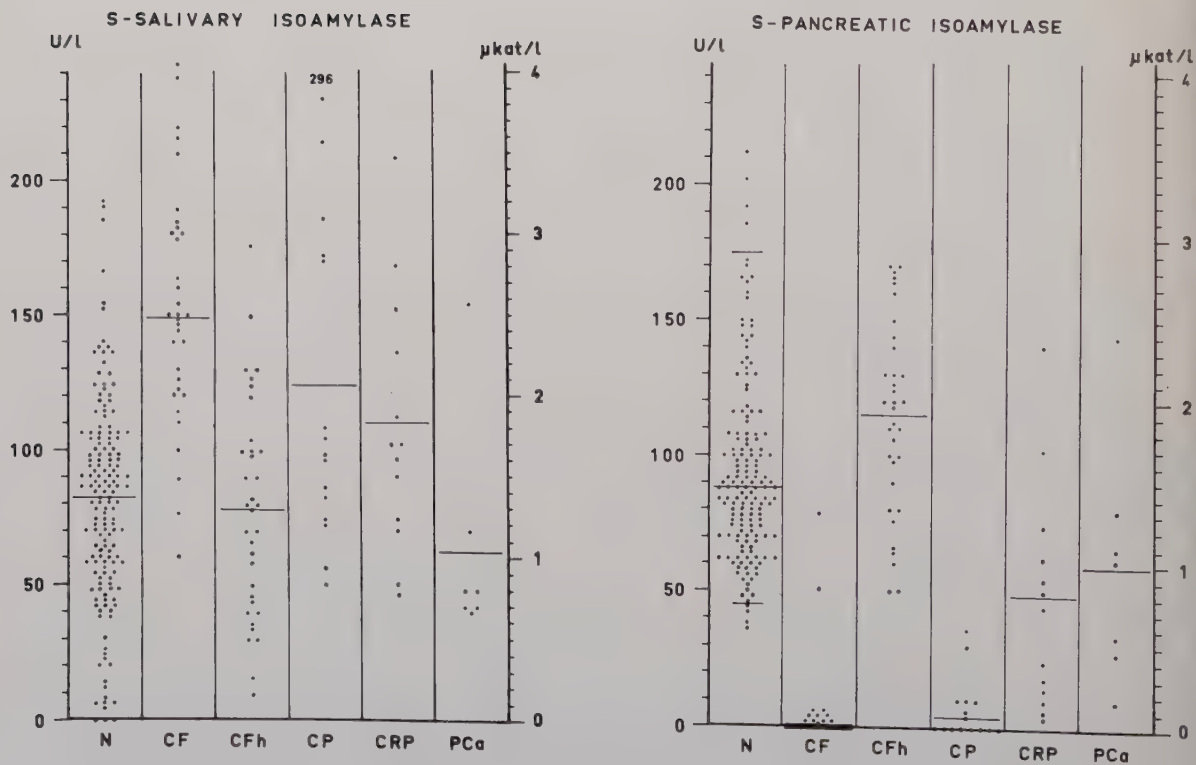


Fig. 2

activities did not vary with sex and the diurnal variation of the serum isoamylases was negligible in persons without pancreatic disease, nor was there any variation related to meal.

Clinical studies

Maxillo-facial surgery (II)

Serum isoamylase was determined in patients undergoing maxillo-facial surgery. Minor surgery (e.g. reduction of minor fractures of the mandible or zygomatic bone) did not influence the serum amylase activity or isoamylase distribution. Major surgery (e.g. bilateral sagittal splitting of the mandibular rami, often combined with osteotomy of the maxilla and free bone grafting) resulted in an elevation of the serum amylase activity in about 50 per cent of the patients owing to up to a 10-fold increase of the salivary serum isoamylase, an increase which disappeared within 2 to 4 days. The pancreatic serum isoamylase activity remained unaltered.

Cystic fibrosis (VI)

The salivary and pancreatic groups of isoamylases were determined separately in the serum of 33 patients, aged 3 months to 28 years, with cystic fibrosis (CF), and in 34 CF-parents. Pancreatic isoamylase activity was greatly decreased in patients with CF, and only 2 values from this group fell within the reference range (Fig. 2). Salivary isoamylase, however, was markedly increased (Fig. 2) so that the total serum amylase activity was normal.

In heterozygotes (CFh) the mean activity of the pancreatic serum isoamylase was significantly higher than in the reference group, while that of the salivary isoamylase was within the normal range (Fig. 2). The genetically determined variation of pancreatic isoamylase with doubled main fractions appeared to be more common in CF-heterozygotes

than in healthy blood donors. This finding, however, could not be confirmed in an extended study (51). The increased pancreatic serum isoamylase activity found in the heterozygotes has been confirmed by another group using the same technique (32) and this is the first demonstration of pancreatic involvement in persons heterozygous for the CF-gene. It was concluded that after 1 year of age the test has a positive diagnostic value.

Chronic pancreatitis (VII)

Serum isoamylases were quantitated separately in 15 patients with chronic pancreatitis (CP). All the patients had steatorrhea indicating probably less than 10 per cent of normal pancreatic function. The mean activity of pancreatic isoamylase was 7 U/l (0.12 μ kat/l) in the patients compared with 88 U/l (1.65 μ kat/l) in healthy controls (N) (Fig. 2). Eight of the patients had ~~no~~ detectable pancreatic serum isoamylase and the mean activity in the others was 15 U/l (0.25 μ kat/l). The salivary serum isoamylase activities were moderately increased in the patients, mean 124 U/l (2.3 μ kat/l) (Fig. 2).

It was concluded that determination of the pancreatic serum isoamylase activity is a simple screening procedure that should be useful in the diagnosis of advanced exocrine pancreatic insufficiency.

Chronic relapsing pancreatitis and pancreatic carcinoma (VIII)

Pancreatic isoamylase was determined in serum from 13 patients with chronic relapsing pancreatitis (CRP)(Fig. 2). The serum activity was abnormally low in 7 of the patients. Of the remaining 6 patients, 3 had a serum activity just above the lower normal limit, 50-60 U/l (0.9-1.1 μ kat/l), while 2 had an activity close to the normal mean and 1 had a rather high normal activity, 140 U/l (2.60 μ kat/l). The 6 last-mentioned patients were all chronic alcoholics who had had several attacks of acute pancreatitis before the development of chronic relapsing pancreatitis. In the patients with high activity pancreatic

disease was suggested by an abnormally high activity of a fast migrating pancreatic isoamylase, P_{O} -isoamylase (see below IX). The mean salivary serum isoamylase activity in the CRP-group was slightly higher than in the reference group (Fig. 2).

Seven patients with pancreatic carcinoma (PCa) were studied; the serum activity of the pancreatic isoamylase was subnormal in 3 of them and in the lower half of the reference range in 3. The mean activity of the group was 60 U/l (1.1 $\mu\text{kat/l}$) (Fig. 2). One patient had a rather high pancreatic serum isoamylase activity. In this case a high activity of the P_{O} -isoamylase fractions suggested pancreatic disease. Salivary serum isoamylase activity tended to be low in the PCa-patients (Fig. 2).

Duodenal aspirates were obtained with the Lundh test (30). Samples obtained from healthy persons, patients with pancreatic carcinoma, and patients with chronic relapsing pancreatitis were analysed for trypsin and pancreatic isoamylase. Total amylase determinations were not used because duodenal aspirates are known to be contaminated with salivary amylase (52). A somewhat larger difference in pancreatic isoamylase activity than in trypsin activity was found between normal subjects and patients with pancreatic disease. The regression between the activities of pancreatic isoamylase and trypsin was non-linear; at low levels the amylase activity decreased faster than the trypsin activity. All persons with an abnormally low pancreatic isoamylase activity in their serum also had an abnormally low enzyme level in the duodenal aspirate. Of the patients with pancreatic carcinoma and chronic relapsing pancreatitis and a reduced level of pancreatic enzymes in their duodenum about half were found to have pancreatic serum isoamylase activity above the lower normal limit. This discrepancy could probably be ascribed to the remaining secretory capacity of the pancreas and the occurrence of obstructive lesions

in the pancreas.

Obstructive pancreatic disease (IX)

The activity of the anodally migrating minor pancreatic isoamylase fractions, P_{O} -isoamylase, in serum comprised less than 20 per cent of that of the pancreatic main fraction in patients without radiographically detectable pancreatic disease and in patients with acute hyperamylasemia. Patients possessing higher proportional activity of the P_{O} -isoamylase in serum were found to have radiographic evidence of pancreatic main duct occlusion or stenosis with or without pancreatic cysts or calculi. In these patients, who often had high total pancreatic serum isoamylase activity, the P_{O} -isoamylase fractions might even be stronger than the normal main fraction. The P_{O} -isoamylase disappeared after surgical correction of the occluding process. The P_{O} -isoamylase had a lower renal clearance rate than the normal pancreatic main fraction, as can be seen on comparison of samples 3 and 4 in Fig. 5. The P_{O} -isoamylase could also be demonstrated in pancreatic secretion and contents of cysts (sample 5, Fig. 5) (VIII). The P_{O} -isoamylase may represent a degradation product of the normal main fraction as a result of proteolytic digestion or deamidation. Such a conversion could be demonstrated to occur in vitro on storage of pancreatic juice at room temperature.

INDICATIONS FOR DETERMINATION OF ISOAMYLASE

The present study and other published results suggest the following range of indications for determination of serum isoamylase.

Salivary gland disease. The serum isoamylase pattern may be abnormal in cases of Sjögren's syndrome, salivary duct concretions, neoplastic and inflammatory diseases such as mumps; in the last-mentioned condition the analysis can also disclose any co-existing pancreatic involvement. In cases with salivary gland diseases, however, determination of the serum isoamylase pattern is of minor importance.

Pancreatic diseases. In acute pancreatitis, the correct diagnosis can almost always be made from the clinical picture and the determination of total serum amylase activity, which thus, generally makes isoamylase determination unnecessary. In cases with prolonged hyperamylasemia following an attack of acute pancreatitis the method can be used for detecting development of pancreatic duct obstruction.

In chronic pancreatitis the analysis is useful for detecting severe exocrine insufficiency. In chronic relapsing pancreatitis and pancreatic carcinoma it can be used as a simple screening test, being positive in more than 50 per cent of all cases. Owing to the presence of the P_o -isoamylase it can also reveal other causes of obstruction of the pancreatic duct system and thus sometimes furnish diagnostic clues in jaundiced patients. Following surgical correction of the obstruction in cases of chronic relapsing pancreatitis determination of the serum isoamylase can give a rough estimate of exocrine pancreatic function.

In cystic fibrosis determination of serum isoamylase is of value, although absence of pancreatic serum isoamylase is a normal finding during the first year of life. Demonstrable pancreatic serum isoamylase in such early life argues against the diagnosis. After 1 year

of age the test has a positive diagnostic value. As the determination of total serum amylase activity with the same chromogenic substrate requires only 15 μ l serum (50), a complete serum isoamylase determination can without disadvantage be performed in small children. The increased salivary serum isoamylase activity in cases of cystic fibrosis is not a reliable diagnostic finding. Although the CF-heterozygotes, from a statistical point of view, behave in a specific manner concerning their serum isoamylases, the method cannot be used for detection of heterozygosity.

Malabsorption. In the evaluation of malabsorption syndromes serum isoamylase determination should be included as a screening test of pancreatic function.

Hyperamylasemia. As postoperative hyperamylasemia is often due to a raised serum level of the salivary group of isoamylase, even after abdominal surgery (51), just as it is in hyperamylasemia following endoscopic retrograde cholangio-pancreatography (55), determination of the serum isoamylase is necessary before making a diagnose of "pancreatitis" in such cases. Unclear hyperamylasemia in a patient with abdominal pain is another indication for determination of the serum isoamylase with special reference to the P_o -isoamylase. In patients with painless hyperamylasemia of unknown etiology the test can reveal whether the increased activity is due to a high level of the salivary or the pancreatic isoamylases or possibly to macroamylasemia. Determination of the serum isoamylase can also be valuable for localizing the primary tumor to the pancreatic or salivary glands in cases of a histologically or cytologically verified metastasis from an unknown primary adenocarcinoma.

Determination of isoamylase is also useful for disclosing the amount of salivary isoamylase admixture in duodenal aspirates obtained in connection with pancreatic function studies using the Lundh test combine

with determination of the amylase content of duodenal aspirates.

The determination of amylase clearance (29,66) has been recommended for detecting the origin of an increased serum amylase activity. In none of the above mentioned conditions would it offer any advantage over electrophoretic serum isoamylase determination and it cannot disclose reduced pancreatic serum isoamylase activity or the presence of P_o -isoamylase.

EVALUATION OF THE SERUM ISOAMYLASE PATTERN

Normal variation

As the serum isoamylase pattern reflects the variation of the pancreatic as well as the salivary groups of isoamylases, knowledge of the normally occurring variation of the isoamylases from both sources is essential. A genetically determined deviation from the usual isoamylase pattern in saliva is present in about 15 per cent of the Swedish population (49). Some of the most common saliva isoamylase patterns are depicted in Fig. 3A. It is clear from the figure that the variation comprises both the level and the mobility of the individual fractions. The most common phenotype is shown as Sample 1, followed by some other phenotypes in decreasing order of frequency. Fig. 3B shows the corresponding serum isoamylase patterns. It is obvious that the inherited variation of the isoamylase pattern in saliva is also disclosed in serum with the presence of generally 2 to 4 of the largest fractions. The salivary isoamylase variants of Samples 3 and 4, respectively, contribute with very minute amounts to the serum isoamylases and is the commonest cause of a low salivary serum isoamylase activity.

The faint zone of these 2 samples showing amylase activity anodally to the main pancreatic fraction is the normally occurring anodally migrating fractions of pancreatic isoamylase, which constitutes less than 20 per cent of the activity of the main fraction. A comparison with the salivary isoamylase fraction occupying the zone between the main salivary and the main pancreatic fractions of the other samples of Fig. 4B shows that the anodal pancreatic fraction has a somewhat lower electrophoretic rate of migration than the slow salivary isoamylase fraction. Sample 6 (Fig. 3A) shows a very unusual and very slow salivary isoamylase fraction; in the serum sample (Fig. 3B)

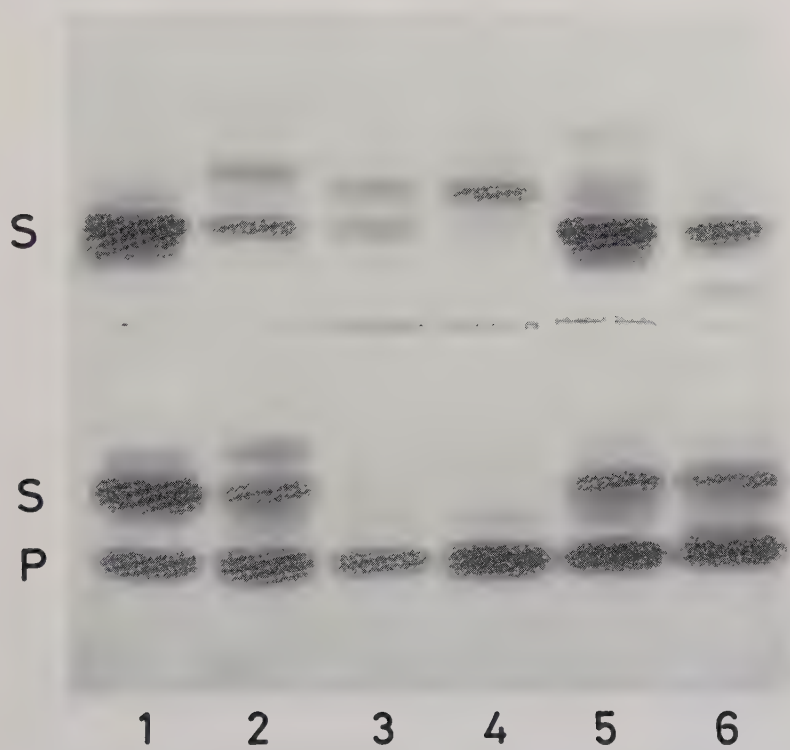


Fig. 3

2

it is seen as a slight anodal broadening of the pancreatic main fraction. Most of the varieties of the salivary isoamylase patterns, however, cause a variation of the zones anodally to the salivary main fraction and do not cause any difficulties in the interpretation of the serum isoamylase patterns.

Genetically determined variation of the pancreatic isoamylase pattern is demonstrated in Fig. 4. Sample 1 shows the normal pancreatic isoamylase phenotype seen in almost 90 per cent of the Swedish population; the pattern is characterized by one heavy main fraction and the additional anodally migrating faint fraction (less than 20 per cent of the main fraction). About 12 per cent of the population, being heterozygous, have an additional cathodally migrating zone showing amylase activity. This zone has a somewhat lower activity than that corresponding to the main fraction of the same individual (Sample 2). The proportions of activity are about 2 to 3, and consequently the total pancreatic serum isoamylase activity is slightly lower than in the commonest phenotype. The individuals homozygous for this gene, about 1 per cent, have such a cathodally migrating main fraction, sample 3. The pancreatic serum isoamylase activity of the last-mentioned phenotype is about 70 per cent of that of the common phenotype. So far, no genetic variants with abnormally fast pancreatic isoamylases have been recorded.

Pathologic variation

The hyperamylasemia of acute pancreatitis is characterized by an increased activity of the pancreatic group of isoamylases (Sample 4, Fig. 4). The increase involves the main fraction(s) as well as one, rarely 2, or more, anodally migrating fractions, whose total activity is at most about 20 per cent of that of the main fraction. The levels reached vary largely, from 100 to more than 10,000 U/l (about 1.5-

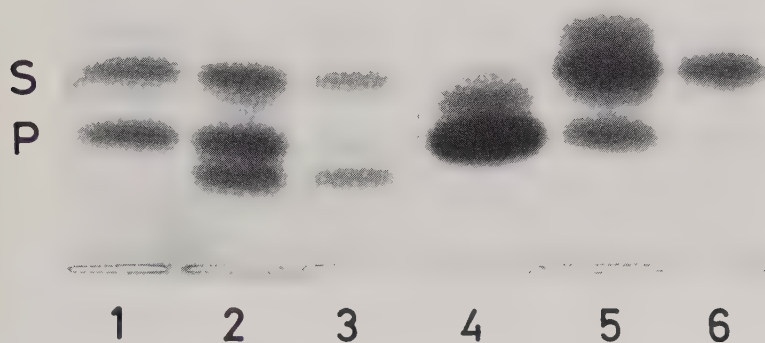


Fig. 4

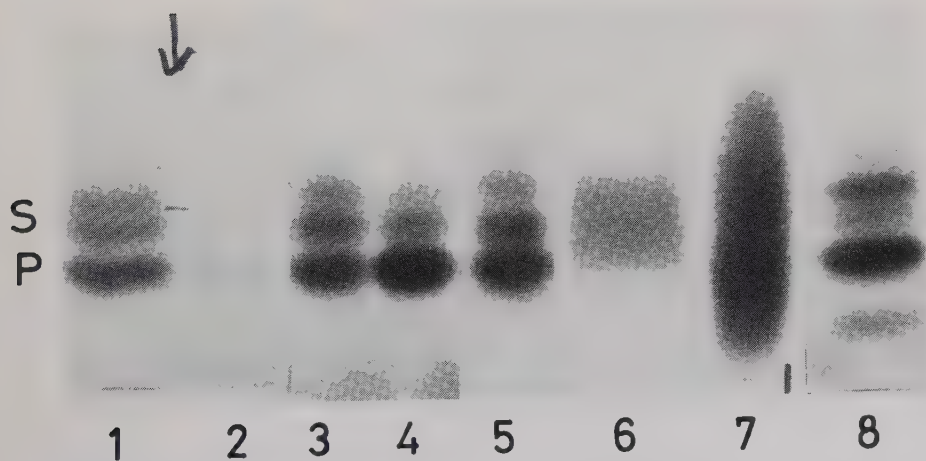


Fig. 5

2

150 μ kat/l), with the functional state of the pancreas and the severity of the inflammation. The same isoamylase pattern showing very high activity is seen also in the peritoneal fluid in these cases. During the following 4-14 days the serum isoamylase pattern gradually becomes normal, the main fraction having a somewhat shorter half time than the additional anodal fractions.

Increased activity of the salivary serum isoamylases gives the typical picture of Sample 5 (Fig. 4). This pattern can be seen after maxillo-facial surgery or in connection with obstructive processes as sialolithiasis and salivary gland tumors, in mumps or in aseptic inflammations of the salivary glands due to various drugs. In mumps complicated with pancreatitis also the pancreatic serum isoamylase activity is increased.

Decreased pancreatic serum isoamylase activity is a typical finding in advanced chronic pancreatitis and in cystic fibrosis (Sample 6, Fig. 4). In such cases the salivary serum isoamylase activity is often increased so that the total serum amylase level is within the reference range.

The pancreatic serum isoamylase activity is reduced in about 50 per cent of all patients with chronic relapsing pancreatitis and pancreatic carcinoma. Normal or increased pancreatic serum isoamylase activity combined with an abnormal isoenzyme pattern characterized by an activity of the anodally migrating pancreatic isoamylase fractions, P_0 -isoamylase (Sample 1, Fig. 5) exceeding 20 per cent of that of the main fraction suggests pancreatic main duct occlusion or stenosis with or without pancreatic cysts or calculi. The patterns possessing P_0 -isoamylase can be seen in chronic relapsing pancreatitis, pancreatic carcinoma, and gallstone disease.

In some cases with chronic relapsing pancreatitis combined with disturbed outflow there occurs a postprandial increase of the pancreatic serum isoamylase activity. A meal or injection of a stimulator of the pancreatic secretion can be used to disclose an obstructive pancreatic disease in some patients.

In patients with a high P_o -isoamylase activity normalization occurs within about 1 week following decompression surgery (Sample 2, Fig. 5) and the residual activity can be used as a rough measure of the exocrine pancreatic capacity. Oral pancreatic enzyme substitution has no influence on the serum isoamylase pattern.

In macroamylasemia the pattern is characterized by a diffusely outlined zone of varying intensity and extension (Samples 6 and 7, Fig. 5). Occasionally some normal salivary and pancreatic main isoamylases can be seen as darker zones in the blurry area. The frequency of macroamylasemia is about 0.1-0.2 per cent in the population and cases have been seen down to the age of one year (51).

Extremely rarely occurring additional zones of starch digesting activity, belonging neither to the pancreatic nor to the salivary isoamylase groups have been recorded in serum (III). In Sample 8 (Fig. 5) the starch degrading activity was not due to amylase as the activity was not eliminated after incubation of the serum sample with rabbit anti-amylase. Such a procedure might be helpful in disclosing starch degrading activity not due to α -amylase.

Since the serum isoamylases are excreted in the urine, some caution is recommended in drawing conclusions from the serum isoamylase patterns in patients with decreased glomerular filtration in which the serum isoamylases are, as a rule, increased.

PROSPECTIVE USE OF DETERMINATION OF ISOAMYLASE

Serum isoamylase determinations may furnish information also about diseases not discussed earlier in this survey. Thus, abnormally high as well as low pancreatic isoamylase activity is relatively common among chronic alcoholics without clinically recognized pancreatic affection (54). According to preliminary results this holds also for patients with infectious hepatitis, and abnormally high serum levels of pancreatic isoamylase have been recorded in Crohn's disease (51) and lung carcinoma (3,61).

Normally the specific serum isoamylases of the female genital tract can be detected in fluid obtained from the cul-de-sac; this is also possible in inflammatory diseases of the lower genital tract, but not in pregnancy or acute salpingitis (53,67). High total serum amylase activity has been found in ruptured ectopic pregnancy (24), but it is still unknown which of the isoamylase groups that is increased in these cases. The physiologic significance of the female genital tract isoamylases is not known, but it might perhaps supply energy to the sperms or ova. If so, a reduced activity may decrease fertility.

From a genetic point of view the study of the salivary as well as the pancreatic isoamylases is of value. As mentioned earlier, the polymorphism of the two isoenzyme systems are determined by alleles situated at 2 linked loci of chromosome 1 (17). The frequency of the genetically determined variants and the large number of alleles of the salivary amylase locus suggest that the systems might be useful as marker systems in linkage analysis as well as in medicolegal and population genetics.

It should also be stressed that the human serum and urine amylase activities are made up of a mixture of individual isoamylases with specific properties and that they are derived mainly from two sources. For instance, the renal clearance of the main salivary isoamylase differ

from that of the pancreatic isoamylases, the half-time in the circulation being about 18 and 12 hours, respectively (51), also, as pointed out earlier, the half-times of the individual pancreatic isoamylases may differ from each other. In research in man and in laboratory animals relevant information and thus, relevant conclusions require specific determination of the individual isoamylases.

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FIGURE LEGENDS

- Figure 1 Age dependent reference ranges for salivary and pancreatic serum isoamylase activity.
- Figure 2 Salivary and pancreatic serum isoamylase activity. N denotes subjects without known pancreatic disease; CF cystic fibrosis; CFh heterozygosity for cystic fibrosis; CP chronic pancreatitis; CRP chronic relapsing pancreatitis; and PCa pancreatic carcinoma. Mean activities of the various groups of patients are indicated as is the reference range. No pancreatic isoamylase activity was detectable in 22 cases in the CF group.
- Figure 3 A: Genetic variation of the isoamylase pattern in saliva. The electrophoretic separation and immunologic detection of amylase was according to I. B: Serum isoamylase pattern of the same 6 individuals. The electrophoretic separation and enzymatic detection of amylase was performed as described in III. Observe the influence of the salivary isoenzymes on the serum pattern. S denotes salivary main fraction, P pancreatic main fraction. Anode above.
- Figure 4 Variation of serum isoamylase pattern. Sample 1: Normal pancreatic serum isoamylase; Samples 2 and 3: Inherited varieties of pancreatic isoamylase; Sample 4: Acute pancreatitis; Sample 5: Major maxillo-facial surgery; and Sample 6: Chronic pancreatitis. The electrophoretic separation and enzymatic detection of amylase was performed as described in III.

Figure 5

Variation of serum isoamylase pattern. Sample 1: Chronic relapsing pancreatitis with duct obstruction and cyst; Sample 2: Same patient as in sample 1, 2 days after decompression surgery; Sample 3: Chronic relapsing pancreatitis; Sample 4: Urine from patient in sample 3; Sample 5: Contents of pancreatic cyst from patient in sample 3; Samples 6 and 7: Macroamylasemia; and Sample 8: Additional zone with starch-digesting activity near the application slit. The electrophoretic separation and enzymatic detection of amylase was performed as described in III.

